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TOPICAL REVIEW

Soil application of high-lignin fermentation byproduct to increase the sustainability of liquid biofuel production from crop residues

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Abstract

When digestates from anaerobic digestion of crop residues are added to soil, a considerable body of information indicates that soil organic carbon (SOC) levels are comparable to those when crop residues are left in the field. This occurs although the amount of digestate added to soil is diminished by digestion and implies that digestion increases the proportion of carbon inputs stabilized as SOC. Here we examine the likelihood and implications of these features being manifested for soil application of high lignin-fermentation byproduct (HLFB) from liquid biofuel production. We show that steady-state SOC levels are much less sensitive to crop residue removal with HLFB return than without it, and provide an example supporting the feasibility of foregoing process energy and coproduct revenue when HLFB is returned to the soil. Informed by this review and analysis, we expect with moderate confidence that long-term SOC levels for soils amended with HLFB from some liquid cellulosic biofuel processes will not be substantially lower than those occurring when crop residues are left in the field. We have high confidence that the economically optimum rate of fertilizer nitrogen (N) application and N_2O emissions will be lower at most sites for HLFB return to the soil than if crop residues were left in the field. We estimate that the per hectare N demand for processing crop residues to liquid biofuels is about a third of the per hectare demand for crop production, giving rise to an opportunity to use N twice and thereby realize cost savings and environmental benefits. These observations support but do not prove the hypothesis that a 'win-win' is possible wherein large amounts of liquid biofuel feedstock can be obtained from crop residues while improving the economics and sustainability of food and feed production. A research agenda aimed at exploring and testing this hypothesis is offered.

1. Introduction

Liquid fuels produced from cellulosic biomass feature prominently in many future energy scenarios, primarily because of their potential to enable carbon (C) neutral or C negative heavy duty transport (Fulton *et al* 2015, Brown and Le Feuvre 2017, van Vuuren *et al* 2018, Field *et al* 2020, Rogelj

et al 2022). In addition, there has been an increased focus on ecosystem C stocks as both a tool and a risk for climate stabilization (Lynd 2017, Ramos and Pressinott 2022). Although a liquid cellulosic biofuel industry was slow to emerge over the last decade (Lynd 2017), replication of profitable industrial facilities has recently begun in Brazil (Ramos and Pressinott 2022).

Crop residues are important feedstocks in many scenarios for production of liquid fuels from cellulosic biomass (Creutzig *et al* 2015, Panoutsou *et al* 2016, U.S. Department of Energy 2016). While use of crop residues for biofuel production avoids concerns related to dedicated use of land for production of bioenergy feedstocks, it potentially affects soil organic carbon (SOC) cycling present in the underlying cropland, as well as nutrient recycling, erosion prevention, and other ecosystem services. The world's agricultural soils have already experienced a median loss of 16% of SOC (25%–61% for a depth of $\leqslant 1$ m) relative to levels before widespread intensive agriculture, representing a total emission to the atmosphere of about 116 Pg C, equivalent to a quarter of cumulative emissions from fossil fuel combustion to date (Sanderman *et al* 2017, Friedlingstein *et al* 2022). Indiscriminate residue removal thus gives rise to concerns related to maintaining soil fertility ranging from caution to alarm with SOC a particular concern (Lal 2004, Cruse and Herndl 2009, Lal and Stewart 2010, Liska *et al* 2014, Johnson 2019).

SOC is the largest reservoir of carbon in the terrestrial biosphere (Smith 2012, Jackson *et al* 2017), is a key determinant of soil fertility (Lal 2004), and has considerable monetized and non-monetized value to society (Lal 2014). It is widely accepted that most persistent SOC has undergone transformations by soil microbes and interacts physically and chemically with soil minerals instead of persisting without transformation due to inherent chemical recalcitrance (Lehmann and Kleber 2015, Basile-Doelsch *et al* 2020). Physical protection within soil aggregates and sorption/complexing to soil minerals or as organometallic compounds are important determinants of SOC storage, and such organic matter persists significantly longer than free particulate SOC (Cotrufo *et al* 2013, Medina *et al* 2015, Dignac *et al* 2017, Basile-Doelsch *et al* 2020, Heckman *et al* 2022). Microbial necromass (dead microorganisms) is a key component of long-lived soil organic matter (SOM) (Cotrufo *et al* 2013, Mazzilli *et al* 2014, Liang *et al* 2019, Buckeridge *et al* 2020, Cui *et al* 2020, Wang *et al* 2021), and there is evidence that microbial carbon use efficiency is a major determinant of SOC storage (Tao *et al* 2023). Below-ground processes involving roots, their exudates, and associated microbiota appear to contribute disproportionately to SOC compared to above-ground biomass (Mazzilli *et al* 2014, Jackson

et al 2017). Considering the importance of microbial transformation, necromass, root inputs, and geochemical characteristics, a strong argument has been made that SOC is an ecosystem property determined by complex interactions between soil's biotic and abiotic components (Jenkinson *et al* 1990, Schmidt *et al* 2011, Dungait *et al* 2012, Lehmann *et al* 2020).

Mechanistic modeling of SOC transformation is a work in progress with significant outstanding uncertainties (Sulman *et al* 2018, Basile-Doelsch *et al* 2020, Hayes and Swift 2020, Lehmann *et al* 2020, Nyang'au *et al* 2022, Noë *et al* 2023). Reasonable predictive accuracy is generally thought to be achieved from models which divide organic matter into slowly and quickly mineralized fractions based on empirical measurements and fitted parameters, provided that models are applied to systems and conditions similar to those used for calibration. The extent to which lignin contributes to, or is correlated with, SOC formation is particularly unclear and indeed contentious (Hall *et al* 2020, Hayes and Swift 2020, Huang *et al* 2023). Hall *et al* (2020) observe that that lignin can lag, lead, or limit the decomposition of litter and SOC. Work of Huang, Hall and colleagues aims to reconcile old and new ideas about the role of lignin in SOC and implicates a growing list of factors impacting which of these outcomes occurs. Such factors include microbeiron interactions (Liao *et al* 2022), solid-phase partitioning (Huang *et al* 2019), oxygen and redox fluctuations (Huang *et al* 2021, Huang *et al* 2019, Hall *et al* 2020), soil geochemical characteristics, and fungal communities (Huang *et al* 2023).

Anaerobic digestion (AD) of crop residues and other lignocellulosic residues to biogas is widely practiced, often with mixtures with manure and other organic material, resulting in solid process residues commonly referred to as 'digestates'. Land application of digestates is the most common fate of digestates (Arthurson 2009, Lukehurst *et al* 2010, Möller and Müller 2012, Dale *et al* 2020, Pastorel *et al* 2021) and has received considerable analysis (Fuchs *et al* 2008, Marcato *et al* 2009, Smith *et al* 2014a, Béghin-Tanneau *et al* 2019, Barłóg *et al* 2020, Nielsen *et al* 2020). Biologically mediated production of liquid cellulosic biofuels also results in a solid postfermentation residue termed 'high lignin fermentation byproduct' (HLFB) by Johnson *et al* (2007). The yield and composition of HLFB from cellulosic biomass-to-liquid fuel processes are different from the yield and composition of AD digestates and also vary among alternative liquid biofuel processes. In contrast to AD of crop residues, for which return of digestates to agricultural fields is the norm, most studies envisioning biological production of liquid cellulosic biofuels assume that HLFB is burned to provide process energy and in many cases exported electricity (Humbird *et al* 2011) as practiced in Brazil

(Chandel *et al* 2019). Other studies considers value-added coproducts derived from HLFB (Schutyser *et al* 2018, Liu *et al* 2019). Return of HLFB to the soil has been considered at a conceptual level (Johnson *et al* 2004, 2007, Johnson 2019) and in a few simulation studies (Pourhashem *et al* 2013, Adler *et al* 2015, Lugato and Jones 2015), but lab data are too scant to be conclusive and to our knowledge field studies examining HLFB return have not been reported.

Here we assess soil application of HLFB from biological production of liquid cellulosic biofuels bringing to bear expertise and insights from three often disparate fields: AD, liquid cellulosic biofuel production, and SOM transformation. Our objective is to explore the possibility that returning HLFB to the field can enable conversion of crop residues to liquid biofuels from land already devoted to food production with at least neutral and ideally positive impacts on the economics and sustainability of agriculture. Nitrogen supply and biological transformation are both a driver and consequence of SOC transformation as well as a key factor impacting agricultural sustainability and economics. Impacts of crop residue removal and HLFB return are thus considered with respect to N management, including N_2O emissions, as well as SOC. Section 2 provides an overview of crop residues, including fate and ecosystem services, production and utilization, and accessibility and sustainable removal levels. The carbon balance and composition of solid residues following biological processing of lignocellulose is considered in section 3. Impacts of adding organic matter to soil with and without biological processing are considered with respect to SOC in section 4, including development of an analytical framework and sensitivity analysis, and with respect to N in section 5. Section 6 articulates the hypothesis that a 'win-win' is possible wherein large amounts of liquid biofuel feedstock can be obtained from crop residues while improving the economics and sustainability of food and feed production. A research agenda aimed at exploring and testing this hypothesis is also offered.

2. Crop residue overview

2.1. Fate and ecosystem services
In soils with regular (e.g. yearly) addition of organic matter, SOC is a mixture of chemically heterogenous compounds including recently added plant material, some of which can mineralize rapidly, and long-lived organic components that can have residence times of centuries and even millennia (Basile-Doelsch *et al* 2020, Shi *et al* 2020, and earlier references therein). When crop residues are left on the soil surface or incorporated into the soil after crop harvest, a large fraction is quickly decomposed. For example, more than 80% of the corn stover left on the land is returned to the atmosphere within 2 years (Buyanovsky and

Wagner 1996, Mazzilli *et al* 2014), and more than 60% of sugar cane straw left on the soil surface is decomposed within a year (Cherubin *et al* 2019). Liang *et al* (2019) report that microbial necromass makes up over half of the SOC in temperate agricultural and grassland soils, and Lugato *et al* (2021) report that mineral-associated organic matter originating primarily as a result of microbial processing of plant inputs represents 70% or more of total SOC. Basile-Doelsch *et al* (2020) estimate that dead organic matter makes up 95% of total SOC, with 60%–99% of this from microorganisms and 1%–40% being particulate organic matter.

Crop residues left in the agricultural field provide ecosystem services including decreased wind and water erosion, maintenance of SOC (which increases water and nutrient retention), weed control, and moderation of soil temperature (Lal 2004, 2014, Wilhelm *et al* 2010, Adler *et al* 2015, Cherubin *et al* 2018). They also provide a source of nutrients which otherwise need to be replaced. For example, Karlen *et al* (2014) found that moderate corn stover harvest (3.9 Mg ha^{-1}) removed 24, 2.7, and 31 kg ha^{-1} for N, P, and K respectively, and that removals increased with increasing stover harvest. Cherubin *et al* (2019) found that by harvesting 12 Mg ha^{-1} of sugarcane straw, the potential N, P, and K removal was 69, 7, and 92 kg ha^{-1}, respectively, representing a nutrient (NPK) replacement cost of US$ 90.00 ha^{-1} (US$ 7.60 per Mg of removed straw). In Asia, where 90% of the world's rice is produced, rice straw contains about 80, 40, and 30% of the potassium (K), N, and phosphorus (P), respectively, taken up by rice (Chivenge *et al* 2020). Soil incorporation of straw can reduce the fertilizer requirement of the subsequent crop and increase SOC, but is not widely practiced currently (Chivenge *et al* 2020). The SOC benefits of rice straw return to the field or paddy may be outweighed, by over 10-fold in some cases, by increased CH_4 emissions when applied under flooded conditions due to anaerobic decomposition (Allen *et al* 2020).

2.2. Production and utilization
Inedible, above-ground parts of annual crops are produced with non-food to food ratios generally in the range of 1:1–1:1.5 for corn, wheat, barley, rice, oats, rye, sorghum, and millet, and 0.25:1 for sugarcane (Lal 1995, Scarlat *et al* 2010), although these ratios are not fixed (Kemanian *et al* 2007). Global production of such crop residues in 2013 was estimated at about 5 billion tons (Cherubin *et al* 2018), corresponding to about 85 EJ of primary energy based on a representative heating value of 17 MJ kg^{-1}. Cherubin *et al* (2018) estimate that global crop residue production increased by 33% in the decade from 2003 to 2013. If production were to continue to increase at this rate in the subsequent decade, global production in 2023 would be about 113 EJ. The estimates of crop residues

by Cherubin *et al* (2018) align well with, and indeed consider, the older estimate of 70 EJ in 2001 by Lal (2005). Most recently, Smith *et al* (2021) estimated the potential energy provision from crop residues produced in 2018 to be approximately 107 EJ. Among annual and semi-annual field crops, corn, wheat, rice, and sugar cane are produced in the largest amounts, occupy the largest land area, and produce the largest amounts of crop residues. According to data compiled by Cherubin *et al* (2018), these four crops account for 76% of global crop residue production in 2013, with Asia producing 47% of total crop resides, America 29%, Africa 6%, and Oceana 2%.

A portion of crop residues is used for fodder, household fuel, construction material, and animal bedding. Perlack and Stokes (U.S. Department of Energy 2011) estimate that approximately 15% of crop residues available at the farm gate for $60/ton will be used for purposes other than bioenergy in the United States in 2030. In Southeast Asia, 30%–40% of total rice straw production is used to feed ruminants (Aquino *et al* 2020). However, the low digestibility of rice straw leads to high yield-scaled methane (CH_4) emissions compared to more high-quality fodder, such as cowpea straw (Allen *et al* 2020), and use of rice straw as fodder has been shown to increase global warming potential by 13% compared to straw burning (Launio *et al* 2016).

A further fraction of crop residues is burned in the field, negating any contribution to SOC and leading to loss of N and P (Van Hung *et al* 2020). Lin and Begho (2022) estimate that between 2000 and 2014 a third of crop residues from rice, wheat, maize, and sugar cane was burned in the field. Local fractions of crops residues burned can be much higher, for example 85% of rice straw in the Baja California region of Mexico (Montero *et al* 2018). The global inventory of Cassou (2018) found that the mass of crop residues burned increased between 1992 and 2012 in ten out of ten regions examined, is expected to remain at about 2012 levels through 2030; China, India, and the United States are the top burners of crop residues, followed by Brazil, Indonesia, and the Russian Federation. Open-field burning of rice straw in South and Southeast Asia is becoming more widespread because of labor shortages, greater mechanization, and increased intensification of cropping systems (Van Hung *et al* 2020). The widespread burning of crop residues is a key factor contributing to poor air quality, with Nepal, Pakistan, India, and Bangladesh being the most affected (Lin and Begho 2022). Diverting these residues for bioenergy purposes would not compete with other markets and would produce air quality benefits.

2.3. Accessibility and sustainable removal levels

The quantity of crop residues that can be practically accessed will be less than the total produced due to multiple factors, including avoidance of ecologically sensitive sites, slope and other terrain limitations, inefficiencies in biomass collection and handling, and availability in quantities sufficient to support a conversion facility with a reasonable feedstock catchment area (Williams *et al* 2015, U.S. Department of Energy 2016). Additional factors are expected to constrain feasible residue supply in the near term with decreasing impact over time if an industry were to develop. These include harvesting equipment, storage and transport infrastructure, seasonal labor availability, and operational considerations reflecting near-term technical challenges for harvesting variable amounts of available residue at field and subfield levels (Huggins *et al* 2014, U.S. Department of Energy 2016).

Comprehensive studies and meta-analyses support transient accrual of SOC over time when 100% of crop residues are returned to the soil for corn (Xu *et al* 2019), sugar cane (Sousa Junior *et al* 2018), and rice (Chivenge *et al* 2020), as well as maize and wheat double crops (Zhao *et al* 2018). Thus, in most systems some fraction of crop residues can be harvested while maintaining SOC at present-day levels. When 100% of crop residues are removed, net loss of SOC is observed for all these crops except rice (Chivenge *et al* 2020). A particular concern associated with the harvest of annual crop residues is loss of SOC due to decreased inputs and soil erosion (Nelson *et al* 2004, Wilhelm *et al* 2007, Adler *et al* 2015, Johnson 2019). For continuous corn, Wilhelm *et al* (2007) found that the retention of corn stover needed to avoid SOC loss was 2.4 times greater than required retention to avoid water erosion for moldboard plow tillage and this increased to 6.2 times greater for no-till or conservation tillage. For corn-soybean rotations, the corresponding figures were 4.5-fold an 8-fold, respectively. While erosion control is a critical constraint for some sites, the literature on allowable crop residue removal for biofuel production focuses primarily on SOC, and we do so here as well.

There is a large body of work aimed at estimating the fraction of above-ground crop residues that can be sustainably removed to produce liquid cellulosic biofuel feedstocks. The vast majority of studies addressing this topic, including all references cited in this section, do not consider the possibility of returning HLFB to the soil. For corn grown in temperate climates, retention of about 6 Mg ha^{-1} of above-ground residues are estimated to be necessary to maintain SOC and avoid erosion (Johnson *et al* 2014), corresponding to about 50% removal based on current above-ground corn yields in the US. A series of studies by the US Departments of Energy and Agriculture estimate the sustainable corn stover resource based on maintaining SOC and crop yields and minimizing erosion at between 150 and 200 × 10^6 Mg yr^{-1}, corresponding to half to two-thirds of total stover

production (U.S. Department of Energy 2011, 2016, Muth *et al* 2013, Bonner *et al* 2014). Most of these studies assume changes to current practice, such as reduced tillage or cover crops, that may compensate for the SOC loss that would occur due to crop residue removal in the absence of such practices. In a study of Southern Sweden, Björnsson and Prade (2021) found that intermediate crops cultivated after harvest of cereals and before the next crop increased SOM at a rate more than 10 times higher than the loss of SOM from removing restrictions on crop residue removal, and that growing such intermediate crops increased the availability of removable straw by 2.5-fold. For sugar cane production in Brazil, return of 6–7 Mg ha^{-1} of sugar cane straw, representing approximately 50% removal, has been recommended based on an extensive series of studies (Carvalho *et al* 2017, Vasconcelos *et al* 2022). Since the above-ground yields of energy cane are about 3-fold higher than sugarcane (Leal *et al* 2013, Junqueira *et al* 2017), it is likely that a lower fraction of energy cane straw needs to be returned to the field. However, this has not been verified experimentally.

Reviewing the literature in 2021, Battaglia *et al* (2021) find that negative impacts of crop residue removal on SOC are less evident for wheat than for corn. Inventories of crop residues in the EU, with wheat the largest contributor, recommend that half to two-thirds be retained in the field (Scarlat *et al* 2010, Thorenz *et al* 2018). In their regional analysis of Southern Sweden, Björnsson and Prade (2021) suggest that high levels of retained crop residues are well-intended but inefficient strategies for SOC preservation and may hinder more efficient measures involving cover crops and conversion of crop residues to chemicals and fuels. For all feedstocks, there is broad agreement that site-specific factors are important to consider when determining the fraction of crop residues that can be sustainably harvested (Meki *et al* 2011, Cherubin *et al* 2018). For example, TenelTenelli *et al* (2021) recommend that sugarcane straw not be removed from sandy soils. Tarkalson *et al* (2009) point out that the allowable fraction of harvestable residues increases with increasing precipitation and yield for wheat and barley, and this is likely the case for other cereals as well. Allowable or recommended fractions of crop residue removal will likely decrease in response to changes in local conditions that accelerate microbial decomposition of agricultural residues and SOC (Andriulo *et al* 1999, Knorr *et al* 2005), but will likely increase in response to continued yield increases.

While concern over removal of crop residues has often been expressed and merits close attention, it is also the case that excessive crop residues are problematic under some circumstances and that partial removal of crop can be accompanied by agronomic benefits. Partial harvest has been proposed for mitigation of negative impacts of high levels of crop residues (Coulter and Nafziger 2008), providing an alternative to increased tillage intensity (Adler *et al* 2015, Vanhie *et al* 2015). Removal of crop residues appears to be an effective way to avoid yield losses from adoption of no-till (NT) management in many settings (Vyn *et al* 1998, Karlen *et al* 2014, Ogle *et al* 2019). An accepted means for reducing erosion compared to conventional tillage (McGregor and Greer 1982, García-Préchac *et al* 2004, Singh *et al* 2009), NT increases SOC at shallow depths although not necessarily in the entire soil profile compared with tilled soils (Baker *et al* 2007, Du *et al* 2017, Cai *et al* 2022). Sindelar *et al* (2015) observe that 'stover removal in continuous corn systems has been shown to have short-term agronomic advantages like increased plant emergence, greater early-season growth, and, subsequently, greater grain production. However, adverse effects of corn stover removal on soil and environmental properties such as SOC declines, expedited nutrient removal, and greater susceptibility to soil erosion are also documented.' Similar considerations apply to sugarcane straw, for which Melo *et al* (2020) found that moderate straw removal resulted in higher per hectare root mass and stalk production compared to both total straw removal and no straw removal.

3. Carbon balance and solid residue composition following biological processing of lignocellulosic biomass

A general C balance for biological conversion of agricultural residues into an organic biofuel is:

$$Y_{\text{Biofuel}}^{\text{C}} + Y_{\text{CO2}}^{\text{C}} + Y_{\text{R}}^{\text{C}} = 1 \tag{1}$$

where $Y_{\text{Biofuel}}^{\text{C}}$, $Y_{\text{CO2}}^{\text{C}}$, and Y_{R}^{C} are the respective dimensionless carbon-based yields of biofuel (e.g methane or a liquid biofuel such as ethanol), carbon dioxide (CO_2), and unreacted solid process residues (digestate in the case of AD, HLFB in the case of liquid biofuel production).

Because separation of biogas from fermentation broth occurs spontaneously and separation of liquid biofuels does not, the authors observe that there is considerably more economic incentive to achieve high carbohydrate solubilization for liquid cellulosic biofuel production than for AD. For the same feedstock, the economically optimal fraction of carbohydrate solubilized will thus generally be substantially higher for liquid biofuel production than for AD, corresponding to a higher value of Y_{R}^{C} for AD than for liquid biofuel production. Whereas Y_{R}^{C} is often reported for AD, fractional carbohydrate solubilization is more commonly reported for liquid biofuel production. These two parameters can however be interconverted for a specified feedstock composition with

assumptions about the biodegradability of various feedstock fractions as developed in the supplemental material.

Based on data from hundreds of farm-scale digesters receiving a mixture of corn stover, cover crops, and manure, a representative material balance developed by the Italian Biogas Consortium features 47% of the fed C remaining in the solid digestate applied to the field. Li *et al* (2016) report solids loss of 49% for AD of corn stover, and 51% of rice straw and wheat straw. Approximately 50% feedstock C loss after attack by anaerobic microorganisms corresponds to carbohydrate solubilization of about 65% (supplemental material) as seen by Liang *et al* (2018) for AD of early season switchgrass and Kubis *et al* (2022) for corn stover utilization by defined thermophilic cultures. For ethanol production from corn stover, the fraction of feedstock C remaining as HLFB following biological conversion is 32.7% in the design of Humbird *et al* (2011) featuring thermochemical pretreatment and added enzymes and 37.3% for the design of Kubis and Lynd (2023) featuring consolidated bioprocessing with cotreatment. The studies of both Humbird *et al* (2011) and Kubis and Lynd (2023) entail about 90% solubilization of non-lignin structural components. For recalcitrant crop residues, we take 0.5 to be a representative value of Y_R^C for AD and 0.35 to be a representative value of Y_R^C for liquid biofuel production, corresponding to about 65% carbohydrate solubilization for AD and 90% carbohydrate solubilization for liquid biofuel production. Lower values of Y_R^C are anticipated for more easily digested feedstocks.

Processes for conversion of cellulosic biomass to liquid biofuels are still under development, with many alternatives being considered. The composition and properties of HLFB have seldom been reported in the literature and can be expected to vary substantially depending on the conversion process and, likely to a lesser extent, the feedstock. For the many biomass pretreatment processes that feature low pH—including processes that involve dilute sulfuric acid, SO_2, steam, or liquid hot water—less than 20% of feedstock lignin is removed and residual solids after biological processing are enriched in lignin (Wyman *et al* 2011) although these processes cause lignin to agglomerate and be chemically altered (Sun *et al* 2015). Lignin from emergent conversion processes featuring mechanical disruption during fermentation *in lieu* of thermochemical pretreatment does not undergo appreciable dissolution or reaction (Balch *et al* 2017). For alkaline pretreatment with sodium hydroxide or co-solvent-enhanced lignocellulosic fractionation (Bhalla *et al* 2019), lignin solubilization can be 85% or higher (Jung *et al* 2018). Residues from biological processing of biomass after such alkaline pretreatments contain little lignin and are not addressed in consideration of HLFB herein.

Looking beyond pretreatment, many liquid cellulosic biofuel conversion processes feature addition of cellulase preparations having very high concentrations of protein, dead microbes (necromass) and biological oxygen demand. Given these factors, there is no basis to assume that properties of HLFB from different conversion processes will be the same, and this includes the fate of HLFB in the soil.

Notwithstanding these uncertainties, salient features may be identified based on both understanding of the processes involved and empirical data. The predominant mechanisms of biologically mediated lignin deconstruction require molecular oxygen (Cagide and Castro-Sowinski 2020, Li and Zheng 2020, Cui *et al* 2022). Consistent with this, carbohydrate is consumed and lignin is very nearly inert for lignocellulose digestion by livestock (Van Soest 1994), AD (Li *et al* 2021), and liquid cellulosic biofuel production processes that produce HLFB (Lynd *et al* 2022). Ranked carbohydrate mass fraction on a dry matter basis from the same feedstock will thus generally be as-harvested feedstock > manure > digested manure > HLFB from liquid cellulosic biofuel production, with lignin fraction exhibiting the opposite trends for most processes. These statements apply to processes occurring under strictly oxygen-free conditions, and do not apply to soil transformations for which oxygen is often present and lignin recalcitrance cannot in general be assumed.

In addition to transformation and mineralization of many organic components of crop residues, transformation of N-containing compounds also occurs during AD and liquid biofuel production. For both AD and liquid cellulosic biofuel production, NH_3 volatilization losses are small during biological processing in closed vessels (Schievano *et al* 2011, Smith *et al* 2014a), nitrification does not occur without oxygen (Firestone and Davidson 1989), and denitrification does not occur since NH_3 is not nitrified to nitrate (Firestone *et al* 1980). Near complete conservation of N is therefore likely for AD prior to field application or post-digestion storage and is anticipated for liquid biofuel production as well. Most agricultural residues have a very high C:N ratio, for example ∼80 (Humbird *et al* 2011) and up to 200 (Kemanian *et al* 2007) in the case of corn stover. Manure has lower C:N ratios as a result of gaseous C respiration, fermentation C losses, despite some animal N retention as protein, and in general has more N present than needed to support AD or microbial processes occurring in soil. AD of manure further decreases the C:N ratio, and much of the N not required to support growth of the anaerobic microbiome is mineralized to ammonium (Gutser *et al* 2005, Möller and Müller 2012). For liquid cellulosic biofuel production, N is generally added to N-poor crop residues to the level necessary to support biosynthesis of the microorganisms mediating carbohydrate

fermentation. As a result, the C:N ratio of HLFB from liquid biofuel production can be expected with confidence to be less than corn stover and greater than manure with or without digestion. These trends are supported by the data in table 1.

For illustrative purposes, we develop here a process-based model applicable to either corn stover processed *via* AD or *via* liquid biofuel production followed by AD of liquid process residues. Results obtained using such a model, described in detail in the supplemental material, are presented in figure 1. As shown therein, about 58% of the C in unprocessed corn stover is structural carbohydrate, about 24% lignin, about 13% extractives, and about 6% other structural components. At 50% of feedstock C remaining in digestate, corresponding to about 65% carbohydrate solubilization and representative of AD of recalcitrant crop residues as discussed above, digestate C according to our model is 48% lignin, 41% structural carbohydrate, 3.9% non-lignin structural components, and 7.5% microbial biomass. At 90% carbohydrate solubilization, corresponding to about 35% of the feedstock C remaining in HLFB and representative of ethanol production from recalcitrant crop residues, HLFB C according to our model is 68% lignin, 16.% structural carbohydrate, 1.6% non-lignin structural components, and 14% microbial biomass.

4. Soil organic carbon-related impacts of adding organic matter to soil with and without biological processing

4.1. Manure with and without anaerobic digestion

Reviewing 11 reports, at least two of which were multi-year field studies, Möller (2015) observes that SOC levels for soil application of manure with and without AD are similar, and that the C losses from biogas production during AD are compensated by decreased C loss after field application. For example, commenting on the comparison of digested and undigested cattle slurry by Fouda (2011), Möller (2015) observed that SOC accumulation was similar although the organic C input for the undigested treatment was nearly twice as high as in the AD treatment. The review of Insam *et al* (2015) also concluded that addition of digested manure does not negatively affect SOC compared to undigested manure applied to agricultural fields. Nyang'au *et al* (2022) found that adding a second AD step prior to soil application of digestates increased the fraction of pre-digestion feedstock (75% cattle manure, 25% grass-clover silage) mineralized during digestion. However, long-term retention of predigestion feedstock carbon increased somewhat with addition of the second AD step, indicating that increased stabilization due to added biological processing more than makes up for

the decreased amount of organic matter applied to the soil.

4.2. Agricultural residues and silage with and without anaerobic digestion

When equal masses of digested and undigested agricultural residues are added to soils, several laboratory soil incubation studies observe that digested residues are mineralized more slowly than undigested residues, and that added digestate leads to the formation of a higher proportion of long-lived SOC (Marcato *et al* 2009, Chen *et al* 2012, Schouten *et al* 2012, Thomsen *et al* 2013, Smith *et al* 2014a, Cavalli *et al* 2017, Béghin-Tanneau *et al* 2019, Nielsen *et al* 2020, Iocoli *et al* 2021). A few studies show increased mineralization of agricultural residues after AD compared to undigested residues (Bernal and Kirchmann 1992, Kirchmann and Bernal 1997). Positive priming appears to have been operative in the study of Bernal and Kirchmann (1992). The 3 year field study of Bachmann *et al* (2014) comparing digested and undigested maize silage found no difference in SOM, and the 2 year field study of Erhart *et al* (2014) observed equal or slightly higher SOM levels for digested biomass compared to undigested biomass. In 76 day laboratory experiments involving crop residues from corn, sorghum, wheat, soybean, and sunflower, Stewart *et al* (2015) observed that high-lignin residues were mineralized more completely than low-lignin residues. By contrast, Nielsen *et al* (2020) observed in 178 day incubations that the extent of mineralization of five anaerobic digestates exhibited a strong negative correlation with lignin content, and no correlation of mineralization with cellulose, hemicellulose, or N contents. The main apparent difference between these studies is that the Stewart *et al* study involved undigested agricultural residues whereas the Nielsen *et al* study involved digestates.

Of over a hundred references identified by searches based on 'anaerobic digestion' combined with soil carbon storage, soil carbon sequestration and similar terms, three were found that consider the same starting material with and without AD, normalize results to biomass prior to biological conversion, explicitly recognize the C lost to biogas during AD, and infer long-term SOC levels from short-term decomposition data. Results from these three studies are summarized in table 2. Thomsen *et al* (2013) evaluated the fate of C in ruminant feed treated differently before addition to soil: no treatment (feed), AD (digested feed), consumed by cattle (feces), and consumed by cattle and AD (digested feces). After fitting net CO_2 release data to a kinetic model, they concluded that the retention in soil of C over decades to centuries appears to be similar whether the initial turnover of plant biomasses occurs in the soil, in the digestive tract of ruminants, in an

Table 1. Composition of crop residues, manure, digested manure, and high-lignin fermentation byproduct (HLFB).

Component	Basis	Corn Stover	Wheat Straw	Rice Straw	Sugarcane Bagasse	Sugarcane Straw	Cattle Manure	Digested Cattle Manure	HLFB from Liquid Cellulosic Biofuels
Water	As harvested or processed weight %	20[d]	10.4[e]	24[e]	5[m]	30–60[k]	91–95[g,i,l,n]	91–94[g,i,n]	44[d]
Carbohydrate[a]	Dry weight %	60[d]	64[e]	60[e]	69[h], 70[f]	65[h], 67[f]	35–40[j,l,n]	20[n]	7.2[d], 6[b]
Lignin	Dry weight %	15.8[d]	17.5[e]	15.6[e]	22.4[h], 27.8[f]	24.5[h], 24.8[f]	13–20[j,l,n]	16[n]	50.5[d], 59[b]
Ash	Dry weight %	4.9[d]	7.1[e]	18[e]	2.9[h], 1.0[r]	4.2[h], 4.9[f]	141	—	17[d]
C:N Ratio	Dry weight %	81[d], to 200[c]	64[e]	46[e]	66[m]	70–120[k]	11–12[j,i,n]	7.0[j,i,n]	15[d], 30[b]
% Inorganic N	NH3 for manure and digested manure; Urea for HLFB	—	—	—	—	—	41–53[g,i,n]	41–58[g,i,n]	Very low

[a] The sum of all carbohydrate species reported—in some cases cellulose + hemicellulose, in some cases separately reporting glucan, xylan, galacatan and arabinan.

[b] (Johnson *et al* 2007).

[c] (Kemanian *et al* 2007).

[d] (Humbird *et al* 2011).

[e] (Bakker *et al* 2013).

[f] (Barros *et al* 2013).

[g] (de la Fuente *et al* 2013).

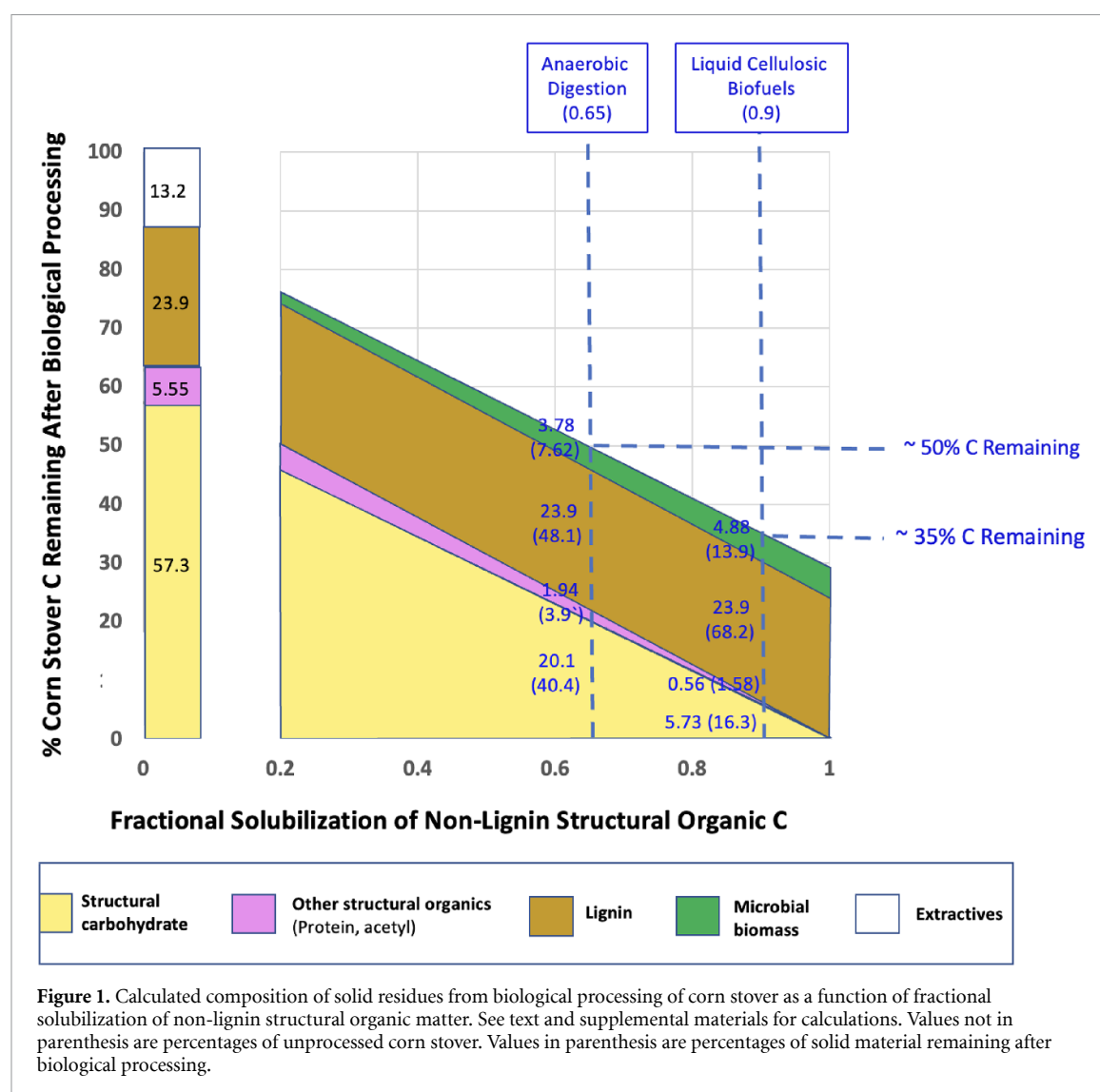[h] (Szczerbowski *et al* 2014).

[i] (Insam *et al* 2015).

[j] (Li *et al* 2016).

[k] (Carvalho *et al* 2017).

[l] (CaCavalli *et al* 2017).

[m] (Xu *et al* 2021a).

[n] (Häfner *et al* 2022).

Figure 1. Calculated composition of solid residues from biological processing of corn stover as a function of fractional solubilization of non-lignin structural organic matter. See text and supplemental materials for calculations. Values not in parenthesis are percentages of unprocessed corn stover. Values in parenthesis are percentages of solid material remaining after biological processing.

anaerobic reactor or in a combination of the latter two. Smith *et al* (2014a) used the Roth-C model with parameters based on soil incubation experiments to estimate long-term soil C levels for leaving agricultural residues in the field compared to various crop residue management strategies including AD. Soil C levels for AD with return of residual bioslurry to the soil were found to be about the same as leaving residues in the field without harvest, whereas removal of crop residues without returning bioslurry resulted in declining SOC. Béghin-Tanneau *et al* (2019) evaluated mineralization of digested and undigested maize silage as distinct from organic matter present in the soil prior to soil amendment. After a 178 day soil incubation, 91% of the C present in the undigested maize silage was mineralized leaving 9% in the soil. For AD, 64% of the C present in maize silage prior to digestion was lost to biogas and an additional 15% was lost during soil incubation, leaving 21% in the soil. For undigested silage, a positive priming effect led to net loss of soil C equal to 4% of the added maize

silage C—that is, lower SOC than without addition of maize silage. For digested silage, a negative priming effect increased the net increase in SOC to 27% of the added maize silage carbon. Iocoli *et al* (2021) also observed that application of digestate was accompanied by a negative priming effect and resulted in net soil C immobilization. Allowing for the loss of over 50% of feed C during AD, estimated SOC levels at the longest times considered are greater with AD than without it for two of the three studies considered in table 2, and are about the same for the third study. It may be noted that the C loss observed during AD of the feeds considered in table 2 is higher than generally observed for lignocellulose-rich agricultural residues of the type considered for liquid biofuel production.
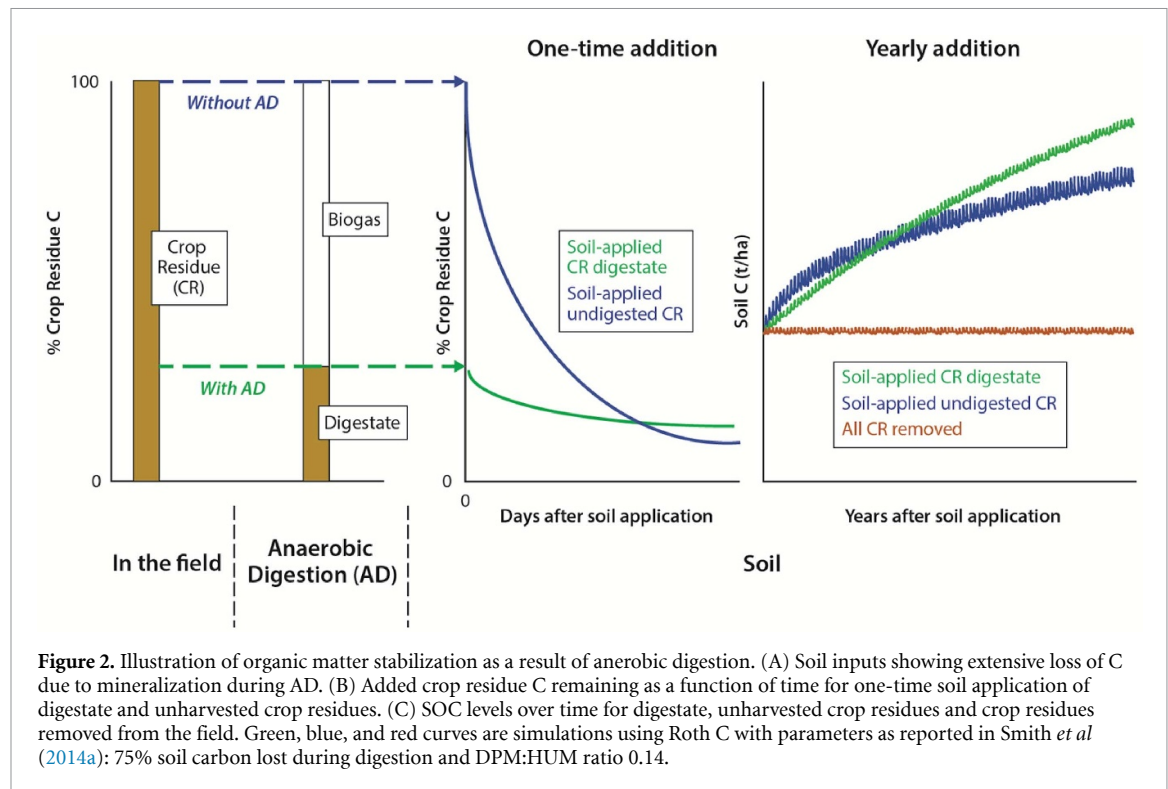
The experience of the Italian Biogas Consortium (Conzorzio Italiano Biogas, CIB), exemplifies benefits of incorporating AD with field application of digestate as part of an integrated management system. In response to a favorable tariff for on-farm electricity production, members of the CIB have over the last

Table 2. Carbon accounting for selected studies of soil application of organic materials with and without anaerobic digestion.

Study	Starting material	Anaerobic Digestion?	Soil incubation or extrapolation time, t	Fraction Starting C Lost			Fraction Feed C Remaining in the Soil at t		Steady-State SOC with & without AD
				Digestion	In the soil	Total	Without priming	With priming[b]	
Thomsen *et al* (2013)	60% maize silage, 21% alfalfa, 18% rapeseed cake, supplemented with basic minerals. C:N ratio 17.6. Neutral detergent fiber 49.3%, lignin 4.3%	No	245 d	—	0.76	0.76	—	0.24	'similar'
		No	1–2 years	—	0.86	0.86	—	0.14	
		Yes	245 d	0.8	0.08	0.88	—	0.12	
		Yes	1–2 years	0.8	0.08	0.88	—	0.12	
Smith *et al* (2014a)[a]	Organic residues available in Sub-Saharan Africa.	No	300 years	—	—	~1.0	—	~0	6.5 ± 0.6 t ha^{-1}
		Yes	300 years	0.69–0.80	0.2–0.31	~1.0	—	~0	8.0 ± 2 t ha^{-1}
Béghin-Tanneau *et al* (2019)	Maize silage. Mixed with liquid dairy manure in the case of AD	No	178 d	—	0.91	0.91	0.09	−0.04	—
		Yes	178 d	0.64	0.15	0.79	0.21	0.27	

[a] Extrapolation using the RothC model assuming average annual air temperature is 20 °C, average soil water content in 25 cm soil is 50 mm, % clay content is 20 g of clay in 100 g soil, field capacity is 80 mm per (25 cm soil), wilting point is 20 mm per (25 cm soil), soil pH is 7, soil salinity (EC 1:5) is 0 dS m^{-1}, average DPM:RPM ratio of plant inputs is 1.44, DPM:HUM ratio of bioslurry is 0.05–0.15, and IOM in bioslurry is 0% after Smith *et al* (2014a). Steady-state values are based on annual addition of organic residues throughout the simulation period.

[b] Thomsen *et al* (2013) did not explicitly consider priming, but offered evidence that it is small in their study. Béghin-Tanneau *et al* (2019) differentiated exogenously added organic matter from SOC based on ^{13}C abundance. The negative value for undigested maize silage arises because the increased mineralization of carbon originating from the soil due to priming is larger than the amount of residual carbon originating from the added silage.

Figure 2. Illustration of organic matter stabilization as a result of anerobic digestion. (A) Soil inputs showing extensive loss of C due to mineralization during AD. (B) Added crop residue C remaining as a function of time for one-time soil application of digestate and unharvested crop residues. (C) SOC levels over time for digestate, unharvested crop residues and crop residues removed from the field. Green, blue, and red curves are simulations using Roth C with parameters as reported in Smith *et al* (2014a): 75% soil carbon lost during digestion and DPM:HUM ratio 0.14.

15 years implemented AD with soil application of both solid process residue and liquid digestate (Dale *et al* 2016, Valli *et al* 2017). Combining NT agriculture with soil application of digestate from crop residues, crops grown during the winter on land that was formerly left fallow, and application of manure, CIB farmers simultaneously realize multiple benefits compared to practices in the absence of biogas production. These include increased per hectare maize yields, SOC, and per hectare revenue, decreased fertilizer inputs, N_2O emissions, and use of pesticides and herbicides, and cumulative production of 1.4 GW of electricity (Dale *et al* 2016, Valli *et al* 2017).

4.3. Increased long term stabilization of organic matter following anaerobic digestion

As presented above, all empirical studies known to us involving manure, crop residues and silage indicate that soil application after AD results in SOC levels comparable to what would occur if crop residues were left in the field. This occurs although the amount of C added to the soil is diminished by digestion when crop residues are converted to biogas, e.g. by 2- to 5-fold, and implies that AD increases the efficiency of long-term SOC stabilization. The mechanistic basis for this increased efficiency is not entirely clear but is consistent with the general notion that the effectiveness of microbial conversion at stabilizing organic matter is comparable whether this conversion occurs in a digester or in soils. These trends are illustrated in figure 2. Substantially less C is added to the soil with AD than for crop residues left in the field (figure 2(A)). Once added to the soil, undigested crop residues added at a given time are initially mineralized very rapidly, eventually reaching lower levels of SOC than more slowly mineralized digestate (figure 2(B)). SOC levels for yearly addition of crop residues (figure 2(C)) are similar for digestate and undigested crop residues but are much lower when all crop residues are removed.

4.4. Agricultural residues with and without liquid cellulosic biofuel production

Soil amendment with HLFB from liquid cellulosic biofuel production has received much less study than soil amendment with anaerobic digestates. In initial studies of soil application of residues from corn stover conversion to ethanol, Johnson *et al* (2004), (2007) concluded that this practice can enhance soil properties but may not negate all problems of crop residue removal. This work did not estimate impacts of HLFB addition on long-term SOC. Cayuela *et al* (2010) observed that a second-generation biofuel byproduct obtained from wheat straw by alkaline pretreatment and enzymatic hydrolysis, but not fermentation, was mineralized more rapidly than unprocessed wheat straw. The composition of this byproduct was not specified but is expected to be substantially delignified and thus to be quite different from HLFB as defined herein. Also, because wheat straw was not fermented, the HLFB would not be expected to contain microbial biomass. To our knowledge there have been no field experiments involving soil application of HLFB from liquid cellulosic biofuel production.

There has been some effort devoted to modeling HLFB return to the soil using models that favor SOC accumulation as the amount of lignin in the organic input increases. In a coordinated pair of studies, Pourhashem *et al* (2013) and Adler *et al* (2015) compared the life cycle greenhouse gas (GHG) emissions and cost for three management options for HLFB from a conversion process featuring dilute acid pretreatment: land application, combustion at the biorefinery to provide process energy, and combustion away from the biorefinery to displace coal used for electricity generation. Adler *et al* (2015) used DayCent to model SOC dynamics over a 20 year period for a corn-soybean rotation implemented at three sites in the US. Simulation results projected that SOC with corn stover harvest and return of HLFB to the soil would be higher at all three sites compared to a baseline with no residue harvest, and had the added benefit of increased available N. Of the three management options considered, Pourhashem *et al* (2013) found that land application had the highest life-cycle GHG emission abatement (g CO_2e/MJ), the lowest capital cost, and the lowest cost for GHG abatement (\$/ton CO_2) assuming a value of \$50 to \$100/dry ton for HLFB as a soil amendment. They concluded further that GHG emissions associated with trucking solid residue from the biorefinery to the field are very small. When lignin is not burned for power generation, Pourhashem *et al* (2013) found that biogas produced from soluble process residues was sufficient to meet the heat demands of the corn stover-to-ethanol design of Humbird *et al* (2011) featuring dilute acid hydrolysis, added fungal cellulase, and yeast fermentation. Biogas is also sufficient to provide process heat demands for ethanol production from corn stover featuring consolidated bioprocessing (Kubis and Lynd 2023).

Lugato and Jones (2015) used the Century model to estimate changes in SOC resulting from corn stover removal for biofuel production in Europe. Management scenarios considered stover left in the field and incorporated into the soil by successive tillage operations, 30% removal of corn stover, and 90% removal of corn stover with (a) no measures to mitigate soil C loss, (b) a winter rye cover crop, and (c) return of 'biodigestate' (synonymous with HLFB) from cellulosic ethanol production based on dilute acid pretreatment. At 90% corn stover removal, SOC was projected to decrease compared to BAU with and without biodigestate return, but biodigestate return reduced SOC loss by 3-fold. Also at 90% corn stover removal, biodigestate return was found to be more effective at mitigating SOC loss than planting a winter rye cover crop. Modeled SOC loss was comparable for 30% corn stover removal without biodigestate return and 90% corn stover removal with biodigestate return. SOC impacts of biodgestate return were not modeled for corn stover removal rates <90%.

4.5. Analytical framework and sensitivity analysis

Informed by the evidence and fundamental understanding presented above, we develop here an analytical framework and compare alternative strategies for managing a given quantity of above-ground crop residues via two strategies:

No Harvest (NH), in which above-ground crop residues are left in the field;

Harvest, Process, and Return (HPR), in which above-ground crop residues are harvested, processed biologically, and solid byproduct (digestate or HLFB) produced at fractional C yield Y^c is returned to the field.

We consider an unchanging yearly schedule of organic matter input over a sufficient time for SOC to reach steady-state, defined as constant SOC at a fixed time of year. We first address SOC formation from above-ground inputs only, with SOC formed from below-ground processes included thereafter. We assume that below ground C inputs and SOC formation are the same for the two management strategies and thus that differences in SOC formation for these strategies are a function of above ground residue management only.

For the NH strategy, a parameter representing the efficiency of SOC formation, τ_{NH} can be defined as the steady-state SOC pool derived from decomposition of above-ground crop residues left in the field ($SOC_{NH,a}^{ss}$, mass C · area^{-1}, with the *a* subscript denoting above-ground) divided by the annual crop residue C (mass C·area^{-1}·time^{-1}). τ_{NH} corresponds to the mean residence time of crop residue C in the soil under NH management and is a measure of the efficiency of crop residue conversion into SOC

$$\tau_{NH} = \frac{SOC_{NH,a}^{ss}}{\text{Annual crop residue C}}. \tag{2}$$

For the HPR strategy, τ_{HPR} can be similarly defined as the steady-state SOC pool derived from decomposition of HLFB or digestate added to the field ($SOC_{HPR,a}^{ss}$) divided by the annual HLFB or digestate C. τ_{HPR} corresponds to the mean residence time of crop residue C in the soil under HPR management.

$$\tau_{HPR} = \frac{SOC_{HPR,a}^{ss}}{\text{Annual HLFB or digestate C}}. \tag{3}$$

Combining equations (2) and (3), we define R_a, the ratio of steady state C pools derived from above-ground inputs, as

$$
\begin{aligned}
R_a &= \left(\frac{SOC_{HPR,a}^{ss}}{SOC_{NH,a}^{ss}} \right) \\
&= \frac{(\text{Annual HLFB or digestate C})}{(\text{Annual crop residue C})} \cdot \frac{\tau_{HPR}}{\tau_{NH}} \\
&= Y_R^C \varepsilon
\end{aligned} \tag{4}
$$

where $\varepsilon = \frac{\tau_{HPR}}{\tau_{NH}}$ is the relative efficiency of steady-state SOC formation from above-ground organic matter for the HPR and NH strategies and Y_R^C is the fraction of crop residue C remaining after biological processing as defined for equation (1) (section 3).

It follows that $\varepsilon\, Y_R^{Cc} = 1$ for steady-state SOC_{HPR}^{ss} to be equal to SOC_{NH}^{ss}, that is in order for R_a to $= 1$. For example, if half the mass of agricultural residue C remains after digestion, $Y_R^C = 0.5$ and ε must $= 2$ for R_a to $= 1$. If $\varepsilon\, Y_R^C > 1$, then $R_a > 1$; if $\varepsilon\, Y_R^C < 1$, then $R_a < 1$.

As presented above in the discussion accompanying table 2, literature reports involving manure, crop residues, animal feed components and mixtures of these indicate that long-term SOC levels are similar for field-applied digestates produced by AD and for crop residues left in the field. That is, $R_a \approx 1$. For the Thomsen *et al* (2013) study detailed in table 2, for which $Y_R^C = 0.2$, $R_a = 1$ implies that $\varepsilon = 5$. For the Smith *et al* (2014a) study, for which Y_R^C is between 0.2 and 0.31 (average 0.255) and the average steady-state value of R_a is 1.23, the implied value of ε is 4–6.2 (average 4.83). For the Béghin-Tanneau *et al* (2019) study, $Y_R^C = 0.36$, R_a is > 1 over the timeframe evaluated, and the implied value of ε is >2.8. $\varepsilon\, Y_R^C \approx 1$ is consistent with a large body of empirical results in the AD literature as well as the observation of Thomsen *et al* (2013) that long-term C retention in soil is similar whether the initial turnover of plant biomasses occurs in the soil, in the digestive tract of ruminants, in an anaerobic digester or in a combination of the latter two.

Analysis of steady-state SOC levels for the HPR and NH strategies can be extended to consider the contribution of below-ground biomass and a variable fraction of above-ground biomass harvested, *f*, as presented in box 1.

Figure 3 presents R_T, the ratio of steady-state SOC values for the HPR and NH strategies, as a function of *f*, the fraction of above-ground crop residue C harvested, using equation (8) with the assumption that b:a $= 1$. The $R_T = 1$ line applies to any combination of ε and Y_R^C such that $\varepsilon Y_R^C = 1$ as repeatedly observed for AD. The $\varepsilon = 4.8$, $Y_R^C = 0.26$ line corresponds to results of Smith *et al* (2014a). The dashed lines are for $Y^c = Y_R^C$, typical of HLFB production accompanying liquid cellulosic biofuels (section 3), and a range of speculative values for ε from 2 to 4. For liquid cellulosic biofuel production with $Y_R^C = 0.35$, the break-even value of ε is 2.86 with $\varepsilon > 2.86$ resulting in $R_T > 1$, that is higher steady-state SOC for HPR than for NR, and $\varepsilon < 2.86$ resulting in $R_T < 1$. In general, the sensitivity of SOC to crop residue removal is substantially less with digestate or HLFB return than without such return.

Box 1. Derivation of an equation for $\left(\frac{SOC_{ss,HPR}}{SOC_{ss,NH}}\right)$ considering the contribution of below- and above-ground plant matter and a variable fraction of above-ground crop residue harvested and processed.

The total steady state SOC for the NH strategy considering above and below ground contributions can be described by modifying equation (2)

$$SOC_{NH,T}^{ss} = (b{:}a + 1)\,(\text{Annual crop residue C})$$
$$* \tau_{NH} \qquad (5)$$

where b:a is the ratio of below and above ground contribution to SOC formation for the NH strategy at steady-state and the subscript T denotes total (above-ground + below-ground).

For the HPR strategy with a variable fraction of crop residue harvested, the contribution of HLFB or digestate applied to the soil plus unharvested above-ground crop residues to steady-state SOC, $SOC_{HPR,a,variable\,f}^{ss}$, can be described by

$$SOC_{HPR,\,a,\,variable\,f}^{ss} = (\text{Annual crop residue C})$$
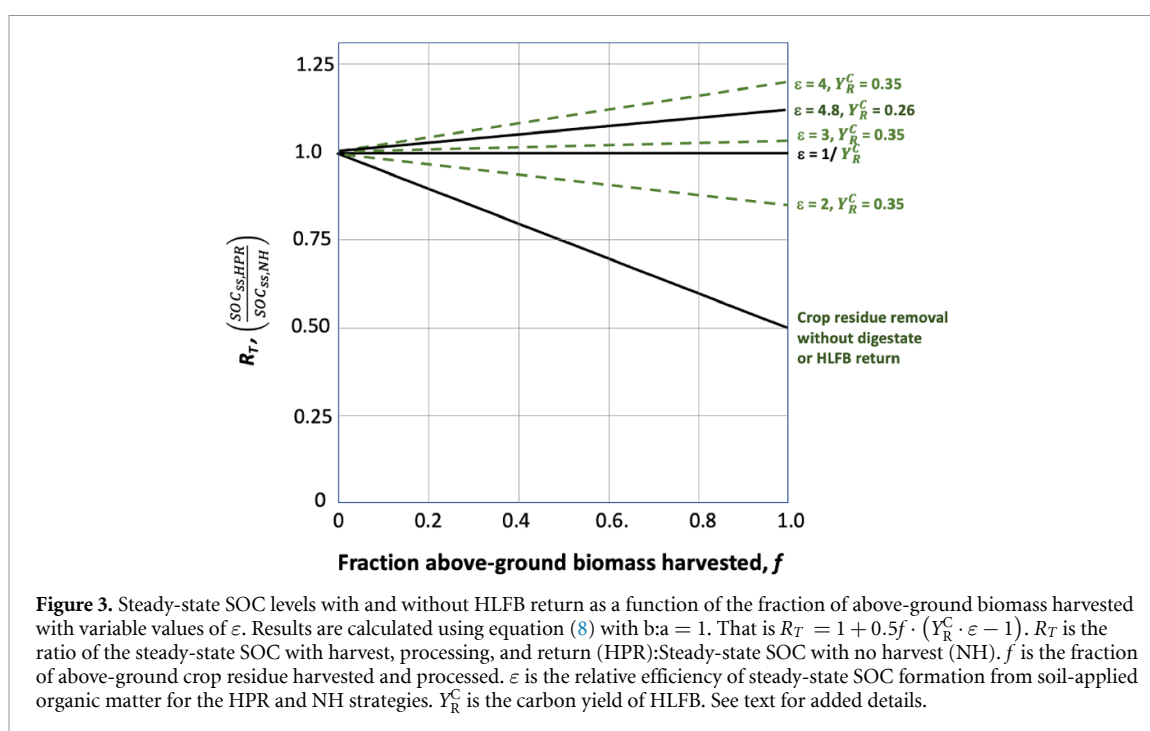$$\times (f \cdot Y_R^C \cdot \tau_{HPR} + (1-f) \cdot \tau_{NH}) \qquad (6)$$

where the $f \cdot Y_R^C \cdot \eta_{HPR}$ term represents SOC from HLFB and the $(1-f) \cdot \eta_{NH}$ term represents SOC from unharvested crop residue left in the field. With below-ground contribution to steady-state SOC the same for the NH and HPR strategies (see supplemental materials), the total steady state SOC for the HPR strategy considering above and below ground contributions can be described by

$$SOC_{HPR,T}^{ss} = (\text{Annual crop residue C})\,(b{:}a \cdot \tau_{NH}$$
$$+ f \cdot Y_R^C \cdot \tau_{HPR} + (1-f) \cdot \tau_{NH}). \qquad (7)$$

Dividing equations (5) and (6) by η_{NH} and substituting $\varepsilon = \frac{\eta_{HPR}}{\eta_{NH}}$,

$$R_T = \left(\frac{SOC_{HPR,T}^{ss}}{SOC_{NH,T}^{ss}}\right)$$
$$= \frac{b:a + f \cdot Y_R^C \cdot \varepsilon + (1-f)}{(b:a + 1)}$$
$$= \frac{b:a + 1 + f \cdot (Y_R^C \cdot \varepsilon - 1)}{(b:a + 1)}. \qquad (8)$$

As for R_a, the condition for R_T to be > 1 is that $Y^c \varepsilon$ is > 1.

Figure 3. Steady-state SOC levels with and without HLFB return as a function of the fraction of above-ground biomass harvested with variable values of ε. Results are calculated using equation (8) with b:a $= 1$. That is $R_T = 1 + 0.5f \cdot \left(Y_R^C \cdot \varepsilon - 1 \right)$. R_T is the ratio of the steady-state SOC with harvest, processing, and return (HPR):Steady-state SOC with no harvest (NH). f is the fraction of above-ground crop residue harvested and processed. ε is the relative efficiency of steady-state SOC formation from soil-applied organic matter for the HPR and NH strategies. Y_R^C is the carbon yield of HLFB. See text for added details.

5. Nitrogen-related impacts of adding organic matter to soil with and without biological processing

5.1. Nitrogen immobilization

Biological transformation and N supply play a central role in forming SOM from agricultural residues, as may be inferred by observing that the 11:1 C:N ratio typical of non-pyrogenic SOM (Khan *et al* 2016) is similar to the 8:1 ratio typical of microbial biomass (Sinsabaugh *et al* 2016) whereas the main components of agricultural residues—cellulose, hemicellulose and lignin—are N free. Most of the N in SOC is in the form of amides (Hedges *et al* 2000). Decomposition of high C:N crop residues in the soil gives rise to substantial N uptake by microorganisms, resulting in decreased availability of mineral N for crops (Aulakh *et al* 2000, Sharma and Prasad 2008, Manzoni *et al* 2010, White *et al* 2014), a process known as microbial N immobilization. Although the N taken up by microbes is not lost to agricultural fields and can become available to crops at times subsequent to fertilizer application, crop residue removal decreases N immobilization, and has been observed to decrease the economically optimal N rate (EONR) in all studies known to us which have examined this. For continuous corn production, Coulter and Nafziger (2008) observed that corn stover removal decreased EONR by 13% compared to no removal. Sindelar *et al* (2015) reported a decrease of >12 kg ha^{-1} of N for NT and >19 kg ha^{-1} of N for strip tillage, and Pantoja *et al* (2015) reported decreases of 22 and 45 kg ha^{-1} for partial and complete residue removal, respectively. These values

are significant relative to the typical application of 188 kg ha^{-1} of N applied to corn on average in Ohio, Indiana, Illinois and Iowa (Xia *et al* 2021). Nitrogen recovery efficiency (N uptake by plants relative to N applied) is higher with corn stover removal than without it at lower fertilization rates, and roughly equal with and without stover removal at higher fertilization rates (Sindelar *et al* 2015). Nitrogen immobilization has also been observed for sugarcane crop residue return (Vieira-Megda *et al* 2015, Rasche and Sos Del Diego 2020), rice (Said-Pullicino *et al* 2014), and wheat (Grahmann *et al* 2014). Rasche and Sos Del Diego (2020) found N fertilizer needs to be 30 kg ha^{-1} higher with sugar cane straw removal as compared to 90% of straw left on the field. However, de Castro *et al* (2021) found only slight N immobilization when sugarcane straw was retained in the field.

5.2. HLFB nitrogen reuse

Microbial conversion of crop residues in liquid biofuel production is expected to require addition of N to support growth of fermentative microorganisms. For liquid cellulosic biofuel production, about 8.2 kg of N is needed per Mg corn stover assuming a cell yield of 0.07 g cell C/g stover C consumed (Supplementary Materials). Nitrogen added during liquid cellulosic biofuel production from agricultural residues is expected to be available for uptake by crops if HLFB were returned to agricultural fields. As a result, we observe that there is an opportunity to use N twice, and thus for production of liquid biofuels and row crops to share the cost of N.

Table 3. Effect of corn stover removal on the economically optimum N application rate and reusable N from liquid biofuel production.

Corn stover removed	Change in the economically-optimum N application rate[a]	Reusable N from liquid biofuel production
%	kg ha^{-1} (% typical application)[b]	kg ha^{-1} (% typical application)[a,c]
50	-22 (-12)	45 (24)
75	-45 (-24)	68 (36)

[a] Based on an application rate of 188 kg N ha^{-1} (Xia *et al* 2021).
[b] From Pantoja *et al* (2015).
[c] See supplemental materials for details.

The magnitude of the change in the economically optimum N application rate due to avoided short-term N immobilization as well as the reusable N from cellulosic biofuel production represents a significant percentage of total N application (table 3). Values for these quantities are expected to be site- and process-specific.

5.3. Nitrous oxide emissions
Nitrous oxide (N_2O) is a greenhouse gas that has ~300 times the global warming potential compared to CO_2 (Smith *et al* 2014b). It is estimated that anthropogenic N_2O emission are increasing and currently amount to 43% of total N_2O emission with agriculture accounting for about two thirds of the anthropogenic emissions (Xu *et al* 2021b). Of GHG emissions associated with N fertilizer production and use, N_2O emissions from soil are responsible for about half of the total and are substantially larger than emissions from fertilizer production (Brentrup *et al* 2016). Emission of N_2O is favored when three conditions coincide: high availability of readily-decomposable organic matter, high availability of inorganic N, and low but not zero availability of molecular oxygen (Firestone and Davidson 1989). Drury *et al* (2021) found N_2O emissions to be higher in one of 3 years with corn stover removal, and Lehman and Osborne (2016) find N_2O emissions to be higher with corn stover removal than without it in the soybean phase of a corn-soy rotation, but differences were minor when averaged through the rotation. Baker *et al* (2014) and Johnson and Barbour (2019) find no change in N_2O emissions accompanying residue removal. These variable results are consistent with corn stover either increasing N_2O formation by providing a source of labile C and consuming oxygen during degradation (Saha *et al* 2021), or decreasing denitrification and N_2O release due to microbial immobilization of inorganic N in a N-poor soils. Most studies of partial or complete removal of sugar cane straw have observed substantially decreased N_2O emissions, particularly in conjunction with application of N fertilizer (Carvalho *et al* 2017, Vasconcelos *et al* 2018, Gonzaga *et al* 2019), although this is not always the case (Gonzaga *et al* 2019, de Castro *et al* 2021). The recent study of Vasconcelos *et al* (2022) found that complete removal of sugar cane straw was accompanied by a 25% reduction in N_2O emissions.

As with SOC, anticipating the impacts of liquid biofuel production with HLFB return on soil N_2O emissions is speculative at this time and can only be made based on inference. Features of HLFB that would be expected to impact relative formation of N_2O compared to NH management include:

- Addition of substantially less organic matter to the soil. As presented in the discussion accompanying figure 1, we estimate about 3-fold less organic matter for liquid cellulosic biofuel production compared to leaving crop residues in the field.
- Because of the economic disincentive to add N to liquid biofuel production processes beyond what is needed for growth of microorganisms, almost all HLFB N is expected to be in organic rather than inorganic form, and soil application of HLFB is expected to be accompanied by much lower inorganic N levels than soil application of manure, digested manure, or inorganic fertilizers.
- Evidence that carbohydrate-depleted, lignin-enriched organic matter after biological processing is less easily decomposable and less stimulatory to N_2O formation than crop residues not subjected to biological processing. The studies presented in table 2 imply that the potential of digestates to yield long-lived SOC is several-fold greater than undigested crops and crop residues, and AD is generally found to reduce N_2O emissions when applied to soils compared to undigested manure (Insam *et al* 2015, Möller 2015). As well, the meta-analysis of Cao *et al* (2021) found that the quality of organic matter is a key determinant of N_2O formation, and in particular that carbohydrates stimulate N_2O formation more than tannins and oxalic acid.

In comparison to leaving residues in the field, crop residue removal with HLFB return to the soil is expected to result in less organic matter added to the field, less N_2O formed per organic matter added, will provide economic motivation to add less N to the field and in any case is expected to involve lower levels of inorganic N. All of these factors favor reduced N_2O formation.

Table 4. Anticipated results of liquid cellulosic biofuel production with HLFB return to the soil.

Feature	Anticipated Outcome[a]	Rationale	Confidence
SOC	Similar	Analogy to well-established results for anaerobic digestate	Moderate Awaits lab and field testing Likely impacted by conversion process features
Economically optimum N application (kg ha^{-1})	Less	Avoided N immobilization	High Magnitude likely site-dependent
Unit cost of N ($/kg)	Less	Opportunity for farmers and biofuel producers to reuse N, share costs	High at small scale Cost penalty at increasing scale to be determined Awaits detailed analysis of HLFB return logistics
N$_2$O emissions	Much less	Lower soil-applied C and inorganic N	High

[a] Compared to leaving crop residues in the field.

6. A potential food/fuel win-win and research agenda

A large body of evidence indicates that soil application of solid digestate from AD results in SOC levels comparable to what would occur if crop residues were left in the field (section 4.3). This occurs although the mass of digested carbon applied to the field is 2- to 5-fold less than the original crop residue C, and implies that the relative efficiency of forming SOC from crop residues—e.g. as represented by ε as defined in section 4.5 is 2–5-fold higher for soil application of digestates relative to undigested crop residues. Compared to unprocessed crop residues, digestate has a lower fraction of carbohydrate and higher fractions of lignin and microbial biomass. Compared to anaerobic digestate, HLFB from the same feedstock is expected with a high degree of confidence to have a yet lower fraction of carbohydrate and a higher fraction of lignin (figure 1). The relative production of microbial biomass in liquid biofuel production and AD is of particular importance given the central role of microbial biosynthesis and organic matter transformation in forming SOC (section 2.1). For compelling economic reasons related to product recovery (section 3), the fraction of crop residue carbohydrate subjected to microbial biosynthesis and transformation is in general higher for liquid biofuel production than for AD. Many thermochemical pretreatment processes proposed for liquid biofuel production modify lignin, e.g via physical and chemical condensation, in ways that are expected to increase recalcitrance to subsequent biological attack. Clearly, HLFB return diminishes the sensitivity of SOC levels to crop residue removal compared to crop residue harvest and liquid biofuel production without HLFB return (figure 3). Given that liquid biofuel production from crop residues converts about a third of crop residue C to HLFB C (section 3), equal SOC

levels for soil application of HLFB and leaving crop residues in the field requires that the relative efficiency of SOC stabilization be about three-fold higher for HLFB than for undigested crop residues. This relative efficiency, corresponding to the parameter ε, has not been systematically evaluated for HLFB. However, an ε value of 3 is well within the range seen for digestate. Based on these considerations, we expect with moderate confidence that long-term SOC levels for soil application of HLFB from some liquid cellulosic biofuel processes will not be substantially lower than for leaving crop residues in the field, and with high confidence that achieving this outcome will be affected by conversion process features that have to date received little consideration relative to processes in the soil.

We have high confidence that the economically optimum N application rate will be lower for soil application of HLFB compared to leaving crop residues in the field, although the magnitude of this effect will be site-dependent. Because the per hectare demand of N for processing crop residues to liquid biofuels can be a third or more of the per hectare demand for crop production (table 3), there is a substantial opportunity to use N twice, first in biofuel production and again when HLFB is applied to the field, and to realize cost savings thereby. This opportunity can likely be realized for small scale biorefineries, but the cost penalty of returning HLFB to cropland at increasing scale has yet to be determined. We expect with high confidence that N$_2$O emissions will be lower for soil application of HLFB compared to leaving crop residues in the field.

Our expectation that SOC will not be substantially lower for HLFB compared to leaving crop residues in the field is based on empirical observations of SOC stabilization in analogous AD systems but is not strongly supported by theoretical arguments in large part because deterministic understanding of SOC dynamics is still a work in progress. By contrast,

Table 5. Key elements of a research agenda pursuant to evaluating liquid cellulosic biofuel production from crop residues with HLFB return.

I. Lab, field, and modeling studies aimed at comparing harvest, process and return (HPR) and no harvest (NH) management, as defined in section 4.5

(a) Empirical data for SOC levels and climate-important soil emissions (N_2O and CH_4) for a range of cropping systems, soil types, and climate regimes.

(b) Understanding the impact of conversion process features, resultant HLFB properties, and field management practices on SOC levels and climate-important soil emissions.

(c) The degree to which N and other plant nutrients in HLFB can meet crop requirements and substitute for other fertilizers.

II. Process, technoeconomic, and life cycle analysis of liquid cellulosic biofuel production processes featuring various fates of HLFB including combustion, value-added coproducts, and soil application, including:

(a) Options for meeting process energy requirements if HLFB is used for other purposes.

(b) Impact of lost revenues from HLFB-derived coproducts in relation to the value of HLFB as a soil amendment.

III. Landscape-scale analysis

(a) Economic and environmental benefits and costs of land-applying HLFB, and

(b) How these vary with scale of the production facility and the feedstock catchment area.

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our expectation that soil application of HLFB will result in lower N_2O emissions is based on what we believe to be consensus understanding that little N_2O will be formed when degradable organic matter and inorganic N are at low levels. Anticipated results of liquid cellulosic biofuel production with HLFB return to the soil compared to leaving crop residues in the field are summarized in table 4.

These observations support but do not prove the hypothesis that a 'win–win' is possible wherein large amounts of liquid biofuel feedstock are obtained from cropland while not sacrificing SOC and improving the economics and sustainability of food and feed production. Confirming this hypothesis would be a major development. By way of illustration, conversion of half of the 100 EJ of global crop residues produced annually (section 2.2) would result in ∼25 EJ of liquid fuel depending on the process (Laser *et al* 2009). This may be compared to about 15 EJ of fuel used by the global aviation sector (International Energy Agency 2021), for which biofuels are widely regarded as a leading low-C option (Fulton *et al* 2015, Davis *et al* 2018). Global production of transport biofuels was about 4 EJ in 2022 (International Energy Agency 2023). Testing the 'win–win' hypothesis articulated here is a high priority in light of the climate crisis, the importance of biofuels for climate stabilization and the extent to which a perceived food

vs fuel conflict has impeded support for biofuels to date. Key elements of a research agenda are presented in table 5.

As noted in II.(a) and II.(b) of table 5, returning HLFB to the soil implies foregoing HLFB-derived generation of process energy and coproduct revenues. Process heat can in principle be obtained from a variety of low-C sources, including solar thermal, renewable natural gas, and biomass—e.g. wood chips (Moreira *et al* 2020), sugarcane straw (Moraes *et al* 2016). For ethanol production from corn stover *via* projected mature technology, biogas produced by AD of process wastewater was found to be more than sufficient to provide process heat requirements without burning HLFB (Kubis and Lynd 2023). Converting HLFB to fuel pellets instead of cogenerating electricity resulted in shorter investment payback periods, improved economic feasibility at small scale, and increased mitigation of greenhouse gases (Lynd *et al* 2017). Analysis presented in the supplementary materials modifies the process model of Lynd *et al* (2017) to examine the impact of soil application of HLFB in lieu of pellet production and export for sale. Even with the conservative assumption that HLFB has zero net value as a soil amendment, we find that the cost penalty associated with foregoing pellet revenue is not prohibitive and indeed is much smaller than the cost savings of foreseeable process improvements. These considerations support the proposition that neither process energy provision nor coproduct revenues are show-stoppers with respect to the feasibility of liquid cellulosic biofuel production with HLFB returned to the soil.

The concept of utilizing HLFB as a soil amendment is analogous to the biochar concept. Both HLFB and biochar are co-products of lignocellulosic conversion to biofuels and bioenergy. Biochar is sufficiently recalcitrant that it supports increased soil carbon storage even after accounting for losses during conversion (Lehmann *et al* 2006), and it is also known to have value for nutrient management and control of N_2O emissions (Joseph *et al* 2021, Kaur *et al* 2023). A growing body of field research suggests that biochar also has small but largely positive effects on crop yields in intensively-managed temperate systems (Jeffery *et al* 2017, Schmidt *et al* 2021). The relative feasibility of biological conversion of cellulosic biomass with HLFB returned to the soil and thermochemical conversion with biochar returned to the soil depend on process economics, which are moving targets since conversion technologies are under development. Increased future attention to soil application of HLFB would appear warranted since both biological and thermochemical production routes are being considered as sources of fuels for aviation and other heavy-duty applications (Falter *et al* 2020, Lynd *et al* 2022).

The liquid cellulosic biofuel field has proceeded to date largely within a 'refinery' conceptual

framework emphasizing valorization of all feedstock components. While this perspective is important and economic viability is a critical prerequisite for impact, circular material flows and maintaining resource stocks are also relevant in light of sustainability, land use and climate considerations. For biofuel production from crop residues, the (bio)refinery and circular economy frameworks lead us in different directions with respect to HLFB management. It will be important to gracefully reconcile this discrepancy as humanity chooses how to deploy cellulosic biofuels in the sustainable resource revolution our times demand.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Author contributions

LL initiated conception of the paper with initial modeling by A A and input from A A and M K, and had lead responsibility for conceiving, writing and editing the manuscript. A K, C H P, J F, J S, P S, T R and M W provided input, critique, and perspective with respect to transformation of organic matter in soils. S B, and C F provided inspiration and input related to the return of digestate to the soil as practiced by the Italian Biogas Consortium, and with T R

provided input, critique and perspective with respect to A D. L L and M K provided input and perspective with respect to liquid biofuel production. M H was responsible for the analysis presented in figure 3 with assistance from M K. All authors reviewed, critiqued, and approved the final manuscript. The data that support the findings of this study are available upon request from the authors.

Conflict of interest

L L is involved in a cellulosic biofuel company. S B and C F are involved in a biogas company. All other authors declare no conflict of interest.

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